

1 how the inoculant was prepared.

2 In the next study in 1963 three clinical
3 isolates were tested and one shellfish isolate was tested.
4 It was determined that disease was -- disease developed in
5 one of the strains only. When they went back they found
6 that this was actually Kanagawa positive.

7 There wasn't really a dose response in this
8 particular experiment that they were looking at. They
9 just gave one dose and four-out-of-four had diarrhea, and
10 I guess usually if you've got diarrhea you've got some
11 abdominal pain that usually comes along with that.

12 In this particular study the cultures were
13 suspended in milk and then followed by a normal meal.

14 In the third study Sakazaki, a volunteer study
15 with fifteen Kanagawa negative strains, fourteen of which
16 were from sea fish and one was a non-hemolytic mutant from
17 a Kanagawa positive mutant. I'm not sure exactly the
18 total number, but with doses up as high as ten-to-the-
19 ninth, no disease was observed.

20 So here again it brings us back to this concept
21 that we've been talking a lot about today that the KP
22 negative strains do not appear to be that pathogenic.

23 However, there is one report, and this was back
24 in 1971, that disease did occur following the feeding of a

1 non-hemolytic isolate. This actually wasn't a full report
2 of itself, this is actually a personal communication to
3 this author from the previous author. So there's just a
4 section in there of a personal communication from
5 Sakazaki.

6 In this particular report, volunteer
7 experiments, it was observed that six to eight hours after
8 feeding a KP positive strain diarrhea resulted.

9 Eighteen hours after feeding a KP negative
10 strain diarrhea also resulted. In both cases his comment
11 is that the dose was approximately ten-to-the six.

12 To readdress this idea of KP negative strains,
13 Sanyal in 1974 again looked at three KP negative strains,
14 but this time, instead of environmental isolates that had
15 been utilized in the previous experiments, these were
16 three strains that were obtained from clinical cases.
17 This was also a clinical case, but again, this was a KP
18 positive.

19 So in here again with these strains, with doses
20 as high as ten-to-the-ten, no disease was observed in
21 these studies.

22 In contrast to that, one KP positive strain was
23 fed at a dose 200 cells, zero out of four showed any
24 response. When you got up to doses of ten-to-the-five, no

1 real diarrhea, but there were complaints of abdominal
2 discomfort, and then when up to ten-to-the-seventh, two
3 out of four had diarrhea and two out of four had
4 abdominal.

5 So, infecting dose, taking these studies,
6 appears to be somewhere between ten-to-the-five and ten-
7 to-the-seven viable cells.

8 However, there are a lot of uncertainties that
9 must be pointed out when dealing with human feeding
10 trials. Although, as I just suggested, that doses within
11 ten-to-the-five and ten-to-the-six may result in disease,
12 these are studies with very select populations. For the
13 most part you're talking about very healthy study people
14 that are volunteering. So we don't have those possibly
15 susceptible groups that perhaps we've been talking about
16 today already.

17 In most cases these studies are done with
18 gastric acid neutralization. So they're either given
19 bicarb to neutralize stomach acidity or given in milk to
20 perhaps neutralize stomach acidity, but what effect does
21 that have? Essentially you're setting up that person
22 because you want that person to get sick, so you're
23 setting up the intestinal tract.

24 Finally, this food matrix effect, what -- are

1 you presenting those bacteria into the gut? How is that
2 effecting? So you've got a very -- it's a very select and
3 it's a very controlled experiment when you do that.

4 You also have very many uncertainties when it
5 comes to the pathogen. In those previous studies there,
6 as you know, the date of the last one was 1974, so what
7 was the characterization of that strain? The papers don't
8 talk very much about where -- the details of those
9 strains. What is the virulence potential of those
10 particular strains? What was the effect of the growth
11 conditions on virulence? How were those particular cells
12 grown, and what effect did that have on the results that
13 were obtained?

14 So you do have results from human feeding
15 trials. They give you ranges, but again you have to keep
16 those uncertainties in mind and the model is going to have
17 to account for that.

18 So what can we do? What's the future of doing
19 additional vibrio parahaemolyticus feeding trials?

20 For our purposes it would be nice to be able --
21 the most direct approach would be if we were able to spike
22 oysters with the new outbreak strains that have been
23 isolated out there, and take those directly to humans.
24 That may be the most direct answer to some of the

1 questions that we may have.

2 Unfortunately, this is unlikely to be repeated
3 this time. And certainly not by July that this is going
4 to occur.

5 There have been reports, and we talked a little
6 about that earlier, there are reports of sudden death
7 that's been associated with infection with vibrio
8 parahaemolyticus. This is not going to get you through
9 any human use committees. So the prospects of doing this
10 are not likely at all.

11 It comes to -- in addition, the thermostable
12 direct hemolysin has also been reported to have some
13 cardio toxicity. This is where they feel that there is an
14 issue involved.

15 So we're not going to be able to do these and
16 repeat what's been done in the past with some of these
17 strains that we'd like to really work with these days.

18 Where does that leave us? The remaining model
19 that's available are surrogate models. In the absence of
20 additional human data for any particular pathogenic strain
21 or serotype of vibrio parahaemolyticus, the alternate for
22 dose-response modeling is to select an appropriate
23 surrogate bacterial pathogen for which additional dose-
24 response data is either available or can be generated in a

1 short amount of time.

2 Additional information that could be considered
3 would be a low dose extrapolation, perhaps looking at some
4 bio-markers. The role of food matrix on infectivity, and
5 also which strain do you pick.

6 Obviously, the most relevant model would be that
7 of using feedings of human volunteers. But, that may not
8 be again possible. So the other option is what animal
9 models could be used that we could extrapolate information
10 from.

11 Animal models using vibrio parahaemolyticus or a
12 surrogate organism could be used to provide a basis for
13 extrapolating dose-response estimates for humans. Animal
14 models can be used to assess the virulence potential of
15 different strains and serotypes, to study the role of the
16 specific determinate. As I mentioned before, the effect
17 of food matrixes and other environmental factors on
18 virulence could be used to study dose-response
19 relationships.

20 All of this could be done much more readily and
21 much more cheaper (sic) in animals.

22 We may be able to compare VP animal models with
23 other animal models for surrogate pathogens which have
24 feeding, human feeding trial data associated with it.

1 Then trying to tie those into it. We can correlate animal
2 models together and that second animal model can correlate
3 back to a human feeding trial, then maybe we can gather
4 some additional information on dose response.

5 In terms of selecting a surrogate there are
6 several criteria that would be looked at.

7 The first one, taxonomic similarity. It would
8 be nice if the bugs were very closely related, that helps.
9 When you're going to produce inferences to the bug of
10 interest it's nice to know that the bug that you've got is
11 somewhat close.

12 Also, the mechanism or the genetics of the
13 pathogens in terms of their virulence factors, you'd want
14 those to be close. We've talked in the past, Chuck has
15 brought up pathogenicity islands, toxin genes. So you'd
16 like that to be as close as possible.

17 Mechanisms of pathogenicity. Is it an invasive
18 organism? Is it a toxin-mediated organism? Again, you
19 would like to have that as closely as you could.

20 Modes of transmission. The same thing. Is the
21 epidemiology of the organism similar to what we're
22 interested in?

23 So these again are obviously factors we want to
24 look to, and we would also want quantitative data for dose

1 and response should exist for these other surrogates. You
2 want information to be available from them.

3 The next slide gives examples of three surrogate
4 human feeding studies that could be used to model vibrio
5 parahaemolyticus dose-response relationships. They are
6 listed on these slides: vibrio cholera non-01, vibrio
7 cholera 0139 Bengal, and campylobacter jejuni. They
8 include two within the same family, obviously those two,
9 and one that used to be in the same family, campylobacter
10 jejuni that now has moved on to its own family.

11 This slide describes the two studies with the
12 two cholera strains. Both of these done by Glen Morris, I
13 believe, up in Maryland.

14 The first one, in 1990, describes studies with
15 vibrio cholera non-01. This is a serotype associated with
16 gastroenteritis, but does not produce the classical
17 cholera enterotoxin. It's been reported to elaborate a
18 toxin that's related to the V.P. hemolysin. It's
19 ubiquitous in estuarine environments, and is commonly
20 isolated from shellfish, including oysters.

21 In this study they had 23 men, 4 women, ages 18
22 to 34, all of them were pre-treated with sodium
23 bicarbonate. In this study three clinical strains were
24 tested and one out of those three produced disease in

1 these volunteers.

2 You will see that the doses are ten-to-the-five,
3 zero with diarrhea, ten-to-the-six, begin seeing two out
4 of three, ten-to-the-seventh, one out of two. You begin
5 seeing disease in the range that was similar to what was
6 seen with the vibrio parahaemolyticus feeding studies.

7 These were all pre-treated with sodium bicarb
8 before they were given the bacteria.

9 In the second study, again with Morris, vibrio
10 cholera 0139 is more of a classical cholera. It does
11 produce cholera toxin. It has all the same virulence-
12 associated genes, including the toxar (phonetic) regulon
13 (phonetic) the virulence cassette, all the CTX, Ace, Zot
14 (phonetic), all the different ones that they've got. But,
15 it just doesn't have the genes that have the biosynthesis
16 of the 01 antigen.

17 Again, in this study volunteers were pre-treated
18 with sodium bicarbonate to neutralize stomach acidity and
19 actually took nothing for 90 minutes before or after the
20 challenge. So in this case you've actually got them
21 challenged on an empty stomach. That's, I think, to make
22 sure that they take all that they're supposed to take.

23 In this study again at a dose of ten-to-the-
24 four, you have two out of four showing signs of disease.

1 At a dose of ten-to-the-six you have seven out of nine.
2 Here slightly lower doses than what we saw with vibrio
3 parahaemolyticus. And again noting that this non-01
4 probably is much more similar in pathogenicity to vibrio
5 parahaemolyticus than this 0139. But, again here's one of
6 the options that's there.

7 This one just shows the last one, campylobacter
8 jejuni, and as I said, this is a closely related -- was
9 within the family of vibrio at one time and now is closely
10 related.

11 In this study two strains of campylobacter
12 jejuni ingested by 111 adult volunteers in doses ranging
13 from eight times ten to the two, to two times ten to the
14 ninth were tested. You do see that rates increase with
15 dose, but development of illness did not show a real
16 clear-cut dose response.

17 Again this brings back this idea of human
18 feeding studies are very -- they are controlled, but
19 you're dealing not with a uniform population, so
20 variability does come into play.

21 Also in this study with campylobacter jejuni
22 they also fasted for 90 minutes and the challenge was
23 given either in dry milk or they were treated with sodium
24 bicarbonate.

1 I'd like to now turn to what's out there in
2 terms of animal models for vibrio parahaemolyticus that
3 could be used to extrapolate information on dose-response
4 relationships.

5 Models that have been described include monkeys,
6 dogs, rabbits, and mice. Some of these models have dose-
7 response relationship information. Some of these models
8 just deal with virulence potential. So different models
9 we may get different information from them.

10 The first one was that using monkeys. This came
11 out of the same study of the first feeding study that I
12 described in 1963. Animals were fed the same lots that
13 were actually given to those human volunteers.

14 In one of three clinical isolates diarrhea was
15 produced along with vomiting at a dose of ten-to-the-
16 tenth. This was the same strain that produced diarrhea in
17 the human feeding trials. So two other strains within
18 that trial did not produce any disease in humans, and they
19 also did not produce any disease in these animals.

20 No disease was found with one shellfish isolate
21 that was looked at. No real dose-response information,
22 just a model that says, yeah, you can determine virulence
23 using monkeys.

24 Also as part of the same study oral challenge of

1 dogs. In this case no disease was found with five of the
2 clinical isolates that were used. Again, going back to
3 that same human feeding trial. One of two shellfish
4 isolates produced diarrhea at a dose of ten-to-the-tenth.
5 So here you have a model that does allow disease, but less
6 discriminatory in terms of what strains you're seeing
7 disease in.

8 Rabbits have been used for virulence for vibrio
9 parahaemolyticus, and several different rabbit models have
10 been reported.

11 The first one is described as the oral challenge
12 of infant rabbits. In this study one KP positive strain -
13 - well, let me go back.

14 In this particular study the animals were orally
15 challenged and then seven hours after challenge they were
16 sacrificed and they looked for -- bacteremia was assayed
17 and then liver and spleen cultures were done on all
18 animals.

19 The results, one KP positive isolate was
20 positive for bacteremia liver and spleen invasion, and one
21 KP negative isolate was negative for bacteremia liver and
22 spleen invasion.

23 In this case doses again were in the ten-to-the-
24 ninth, the ten-to-the-tenth range.

1 It's interesting to note, and we will actually
2 get back to it a little bit later, that the percentage of
3 positive blood cultures was increased when these
4 particular strains were first passed through animals,
5 gotten out of the animal, and then reintroduced into new
6 animals. The extent of bacterium disease actually
7 increased.

8 This slide shows two other models that have been
9 used with rabbits. The first one is the rabbit ileal loop
10 model and this is something that Chuck alluded to earlier
11 today. This is a in-vivo model, but very set up for
12 infection.

13 In this particular model essentially you
14 anesthetize animals, surgically open them up, remove their
15 intestinal tract, and tie off a series of blind loops
16 within the intestinal tract.

17 You then inject samples into these loops, either
18 live bugs, toxin fractions, whatever you want to test,
19 into these loops. Put the loops back into the animal.
20 Sew up the animal. Have the animal survive another 18 to
21 24 hours. At that time sacrifice, reopen up those, and
22 you look for fluid accumulation within those loops.

23 Essentially what you come up with is you come up
24 with a ratio of the amount of fluid per centimeter of

1 tissue length of the loop and that gives you a relative
2 idea of how much fluid is coming into those loops. These
3 have been extensively used for enteric toxins.

4 The results of these studies have shown that
5 most of the KP positives are positive within this assay
6 and in a study of -- let's see, they used three KP
7 positive strains for this particular study. They had LD
8 50 ranges, which they described as the dose where 50
9 percent of the loops were positive. They had as low as
10 ten-to-the-five cells per loop produced positive. They
11 estimated that positive loops were initiated with doses as
12 low as ten-to-the-two cells per loop.

13 As Chuck mentioned and as the literature does
14 stand firm on, most KP negative strains are generally
15 negative within this assay system.

16 Recently there have been studies with
17 genetically manipulated strains where the TDH genes have
18 been genetically removed, and in this case the mutants are
19 also negative in the assay where the parent was positive,
20 with the ileal loop assay.

21 This final one is an in-vitro assay that's out
22 there, it's called using chambers. This is an in-vitro
23 test system. This is a system in which rabbit ileal loop
24 tissue is mounted in chambers, little chambers, and you

1 add your sample to one side of this chamber. You look for
2 changes in short circuit current that goes through your
3 tissue segment. So you're looking for the ability of your
4 organism or your toxin or whatever to disrupt the
5 integrity of that tissue that you've lined in the wall.

6 This showed positive response in TDH positive
7 isolates only and a negative response with this particular
8 mutant in the same study, and they've also showed positive
9 responses with purified TDH.

10 Finally, we'll just talk about some mice models
11 that are out there that have given some dose-response
12 relationship information.

13 The models that have been reported, IP challenge
14 of adult mice, orogastric challenge of adult mice, and
15 orogastric challenge of infant mice.

16 The next slide describes those studies with IP
17 challenges. These are composite groups where four TDH
18 clinical strains were used. Three TDH negative clinical
19 strains and five TDH negative food strains were used and
20 inoculated in a dose-response fashion.

21 You'll see that within these groups mortality
22 was concomitant with the inoculum size. Death rate
23 appeared to be irrespective of the TDH phenotype in
24 inoculum strains. Mice tolerated the lower doses of ten-

1 to-the-five and ten-to-the six, but administration of
2 doses more than ten-to-the-seventh resulted in sudden
3 increase in the death rate, and by ten-to-the-eight almost
4 all animals were killed upon challenge.

5 LD 50 dose ranges went from about ten-to-the-
6 sixth to ten-to-the-seventh, but no statistical
7 differences were seen between mortalities among the
8 challenged groups. So here's a model where you don't see
9 differences between the TDH negative and the TDH positive.

10 This is a continuation of the same study, but
11 here the challenges were oral. Again a composite of
12 strains, two TDH positive clinical strains, two TDH
13 negative strains. One was a food. One was a clinical.

14 Again, you have doses ten-to-the-seventh, ten-
15 to-the-eight, ten-to-the-ninth, and again as before,
16 you're looking at the number of deaths that occurred.

17 As before, no significant differences were
18 noticed between the challenges dependant on the TDH
19 phenotype.

20 Mortality rates reached about 80 percent by ten-
21 to-the-ninth, and they do show a dose-response
22 relationship.

23 Finally, the last study, just to point out one
24 other model that's available, is the oral challenge of

1 infant mice. In this study one TDH positive clinical
2 strain was isolated and a dose of ten-to-the-seventh
3 showed a positive fluid accumulation within this animal
4 assay.

5 This is just an example that was provided by
6 John Bowers, who is the modeler in the group. This
7 illustrates one possible modeling approach using the
8 orogastric and the intraperitoneal challenge data that was
9 just presented with the mice. This is called a Probit or
10 Probit Regression Model.

11 This model assume that there exists a
12 distribution of sensitivity to the bacteriological agent
13 over the population. I believe more of a normal
14 distribution. It looks at the probability of response. A
15 zero Probit turns out to be -- is the indication of a
16 fifty percent probability of illness. If you move over
17 here you can come up with a LD 50 or an infectious dose.

18 This is just presented as one option that may be
19 available. That some of the animal model can be used to
20 generate data that does have dose-response relationship
21 information.

22 Other statistical models will be evaluated,
23 because this model particularly, I guess, is -- well,
24 differences will be different in different models,

1 depending on if you're looking at the LD 50 or you're
2 looking at low extrapolation doses. So this is just to
3 present that models are out there that can be used, and
4 the data can be incorporated into some of these models.

5 I'd like to turn now to surrogate animal models.
6 Again, with the possibility that these may be able to
7 provide some sort of information that we can use.

8 The first model is going back again to a rabbit
9 model. This is called the removable intestinal tie adult
10 rabbit diarrhea model. For those who are familiar with
11 this and have done a lot of this, I'm not exactly sure who
12 the RITARD is. When you're finished with 30 or so of
13 these you really feel like you've done quite a job.

14 This was done with the same non-01 vibrio
15 cholera strains that were done with the humans, that Glen
16 Morris reported.

17 This is also a surgical model. You open up the
18 rabbits, tie off the cecum, put in a little slip knot to
19 put in sort of a blind pouch. You tie off the intestinal
20 tract. Inject the bacteria, sew up the animal, except for
21 this little slip knot. Let that incubate for four hours.
22 Pull the slip knot and then close up the animal. Again,
23 you're setting up the animal for infection.

24 In this study the severity and extent of

1 intestinal damage was dose dependant. The higher doses
2 resulted in more severe disease that developed faster.

3 Mortality occurred quicker as well in the higher
4 doses. At the ten-to-the-ninth dose all died within 24
5 hours. At the ten-to-the-fourth dose deaths occurred 24
6 hours later.

7 It's interesting to note in this study that
8 rabbits challenged with either of the two strains that did
9 not cause disease in the humans, also did not produce any
10 diarrhea in these rabbits. So the only strain that
11 produced positive results in the rabbit model was the same
12 strain that produced positive results in the human
13 studies.

14 This lends perhaps mechanistics -- this
15 mechanistic similarity lends validity to the use of these
16 models as surrogates.

17 Also used infant mice model. Again, using non-
18 01 vibrio cholera. Results of this are just using sero
19 dilutions showed in LD 50 of ten-to-the-seventh and an SF
20 50, which is what they call a fecal staining. This is the
21 dose that caused death. This is the dose that just
22 resulted in fecal staining. So you have diarrhea but not
23 severe enough to cause death. You see it at a lot lower
24 value than causing death.

1 Again, these two are just put up here as
2 possibilities that may be used to gain information on
3 things.

4 I'd like to go back, in the final segment of
5 this talk, to go back to this disease triangle. I'd like
6 to describe what is known about these factors in terms of
7 vibrio parahaemolyticus. At the same time, hopefully this
8 will be a section where it will raise a lot of the
9 uncertainties that I've just described and that have come
10 up throughout the day.

11 The first one is looking at the pathogen itself.
12 We've talked a lot about the virulence potential of any
13 particular strain. What is the potential of strains and
14 serotypes? Are they all alike? For the most part it's
15 obvious the thermostable direct hemolysin is a major
16 virulence factor.

17 But, as we've talked about, there are cases that
18 are out there that are from TDH negative strains. Are
19 they pathogenic? Or is it, as Bill alluded to, it's just
20 that we're not taking them out of the samples. It's just
21 that our methods of detection are not good enough.

22 There are, as Chuck talked about, certain
23 serotypes that seem to have a predominance of disease.
24 They talked about the 04, the 01, and certainly we heard a

1 lot about the 03. So are there serotypes out there that
2 are hotter than other ones and are those the ones we have
3 to worry about? How do we account for those?

4 Some animal models have shown positive responses
5 with TDH negative isolates. Some of the mice data we
6 showed and then there's some work going on at CFSAN right
7 now with some other mice models and some other strains
8 that indicates that you can see disease with TDH minus
9 strains.

10 There have been reports of Shiga-like cytotoxin
11 in strains of vibrio parahaemolyticus. What is their
12 role? Enterotoxins have also been. Enteroinvasive.
13 There have been tissue culture studies, there have been
14 rabbit studies that have showed invasion of the wall with
15 vibrio parahaemolyticus. What is the role of this in the
16 disease process?

17 We may have -- what's been reported for a lot of
18 pathogens currently, is what's the role of in-vivo
19 passage? What's the role of the host actually turning on
20 virulence genes within the host and thereby increasing
21 pathogenicity?

22 As I mentioned before, in the orally challenged
23 suckling rabbits the percentage of positive blood cultures
24 was increased when strains that had been previously passed

1 in suckling rabbits were used. There seemed to be some
2 turn-on of virulence when they went through these animals.

3 What unknown virulence factor may be expressed
4 only within the human intestinal tract or another site of
5 infection that we have no idea that's occurring?

6 Is there a possibility or a possible role of the
7 oyster in regulating virulence potential? What does the
8 oyster do by itself?

9 This slide, as an aside, I'd just like to
10 introduce you. We've termed jimi jejuni, and this is a
11 campylobacter jejuni. I just want to point out this an EM
12 picture of campylobacter jejuni in the intestines of an
13 animal. We're still working on it, but we really feel
14 when it turns on its antennae and it turns on its eye
15 genes, that this is what really enhances pathogenicity.
16 So, they're out there, we just have to prove them and try
17 to find them.

18 In terms of host, are there certain populations
19 more susceptible? Dr. Buchanan has brought that up a lot.
20 There are examples of underlying disease and how do we
21 have to model that?

22 There are papers that show the production of TDH
23 enhanced by bile acids. So the introduction of bile acids
24 into the growth media and those bile acids that are common

1 in humans have shown four to sixteen-fold increases in
2 TDH. What role does that play?

3 Iron limitation also enhances virulence in
4 certain models. These are in the mouse model. I think
5 this was just production.

6 Also we talked about acid adaptation. Chuck
7 talked a little bit about this, or Andy. It was shown in
8 the same paper that in mice acid adaptation enhances
9 virulence in this particular study. What role do these
10 play in the process?

11 Food matrix again has an effect. We don't know
12 what that is. Enhanced virulence with the addition of
13 mucin. Can the food effect the acid in your stomach so
14 that you lower the acidity that it's supposed to and then
15 increase? Could the food matrix increase bile secretions
16 and influence virulence?

17 These are questions that have to be answered.

18 Finally, the last slide just shows again that
19 there's the uncertainties. Each of the models that were
20 out there and that we discussed. Are there
21 epidemiological approaches? Human trials, animal models,
22 or surrogate models, they all have uncertainties. They
23 all have maybe some pluses, but they also do have
24 negatives, and all of these do have to be taken into

1 account with any model that's going to be developed.
2 Thank you.

3 DR. MICHAEL JAHNCKE: Questions from the
4 committee? Bob?

5 DR. ROBERT BUCHANAN: This one is really off the
6 wall too. Bob Buchanan, FDA. We've heard it mentioned
7 several times during the talk as sort of little side
8 comments that there's a high correlation between raw
9 oyster consumption and beer consumption.

10 DR. DONALD BURR: I think what is an oyster
11 meal? How much butter did they take and how much bread
12 did they eat. How much beer did they drink, and how much
13 alcohol is there?

14 DR. ROBERT BUCHANAN: Is there anything
15 associated with the human response to alcohol that would
16 in some way enhance the likelihood that vibrio would get
17 through the stomach and establish itself in the intestinal
18 tract?

19 DR. DONALD BURR: The model that Glen Morris has
20 developed has looked at some -- was that an alcohol model
21 with mice? They tried sort of spiking mice with alcohol
22 and looking for infectivity. I think it didn't work so
23 well. That was another one that was kind of questionable
24 getting through some of the committees. But, there's not

1 a lot out there. But, I think, as you said, what are they
2 eating a meal in when they're eating their oysters, what
3 else are they taking down?

4 DR. MICHAEL JAHNCKE: Other questions? Thank
5 you very much, Dr. Burr.

6 Our last presenter for this session is Dr.
7 Marianne Miliotis. She will be doing a summary on what
8 was presented today.

9 DR. MARIANNE MILIOTIS: In the next five minutes
10 I will summarize everything that's been said to you since
11 the break this morning.

12 To summarize, the next three slides are a brief
13 outline of a preliminary model we are considering on how
14 we can integrate all the data that you've heard about
15 today.

16 This slide is an input/output distribution
17 structure of the pre-harvest/harvest module. As you can
18 see, all the factors, all of the outside circles that --
19 everything that Chuck mentioned, those are parameter
20 distributions and they are going to serve as our input
21 distributions.

22 These two circles to your right are the VP
23 levels at the time of harvest. Those two circles are the
24 output distributions of this module and will serve as the

1 input distribution of a post-module.

2 Here we've taken all the parameters, time to
3 refrigeration, intervention strategies, characteristics of
4 growth, and those are all our input distributions for the
5 post-harvest module. The output of this is the predicted
6 and observed levels of vibrio parahaemolyticus in the
7 oyster at time of consumption.

8 These output distributions are going to serve as
9 the input distribution of our final module, which is
10 public health. You can see there are predicted VP levels
11 at time of consumption. All of these will enter into our
12 final module, which is distribution of vibrio
13 parahaemolyticus human illness.

14 This is the final output and this is our
15 endpoint. It's at this time, on the assumption that all
16 the sub-populations have the same percentage of
17 consumption of raw shellfish. This is where we'll find
18 the difference.

19 Like other people before us and other risk
20 assessments, we plan to run simulations to develop a model
21 obvious different distributions to serve as the framework
22 to better understand the relationship between vibrio
23 parahaemolyticus illness and all the parameters that have
24 been identified today.

1 So while providing this framework many necessary
2 assumptions will be made. For example, one is assuming
3 that the different sub-populations will eat the same
4 amount of oysters.

5 The temperature data. We have some data, very
6 sparse data from studies conducted in the seventies.
7 People have taken seawater and put it -- inoculated them
8 with both parahaemolyticus, KP positive and KP negative
9 and left them for various times and different temperatures
10 to see the difference. We may have to use that kind of
11 data and assume that's what happens in the sea as well.

12 So in conclusion we hope that this risk
13 assessment will provide a scientific framework for the
14 development of food safety guidance and policy to reduce
15 risk of illness.

16 Hopefully, what we will be able to take this
17 risk assessment to our risk managers and say, here is the
18 data. Together with the ISSC determine principal factors
19 to be considered when developing criteria for the closure
20 and reopening of harvest waters. Evaluate the preventive
21 strategies and reevaluate the current FDA level of ten
22 thousand CFU per gram.

23 These are all the members of our task force, our
24 internal FDA task force. I'd like to acknowledge all of

1 them, the different modules of pre-harvest, the post-
2 harvest. Then we have the public health module. Thank
3 you all.

4 DR. MICHAEL JAHNCKE: Thank you very much. What
5 we'll have now is a committee discussion. We'd like to
6 all the NAC members to the table, and also the presenters,
7 at least for the afternoon session. I'd like to invite
8 all the presenters for today to join us at the table. I
9 think that will make discussions much easier.

10 There are a couple of questions that we as a
11 committee need to consider also. Keep in mind if you have
12 questions on the document itself, which is under Tab 8 of
13 your book, parameter identification for risk assessment on
14 vibrio parahaemolyticus and raw molluscan shellfish. Also
15 keep in mind the three questions that were initially put
16 to us this morning. What other data do we need? Is the
17 scientific approach sound? Are there any comments and
18 suggestions on that?

19 We are now open for comments and questions.
20 Dane?

21 MR. DANE BERNARD: Thank you, Mike. Dane
22 Bernard. I didn't catch in the summary, and it was
23 probably there, but as to the last presentation and the
24 summary, I'm unclear as to how prevalence of those that we

1 think are pathogenic, TDH positive, Kanagawa positive
2 strains, the relatively low percentage, how that's going
3 to be factored into the risk assessment? The last
4 presentation seemed to indicate that there are some
5 indications that maybe that's not the only vibrio
6 parahaemolyticus that we need to worry about, and I think
7 there's always that concern. But, how is that going to be
8 dealt with in the risk assessment? What assumptions are
9 we going to make there? How are we going to use them?

10 DR. MICHAEL DINOVI: Mike DiNovi. As long as
11 you can determine some relationship, linear, curve,
12 whatever, between the virulent strains and the non, you
13 can factor that into the portion that I'm putting out,
14 where you'll get a distribution of doses. If you know of
15 a relationship you would just factor and we'd just change
16 the shape of the curve accordingly.

17 DR. ANDY DEPAOLA: Another possibility is from
18 the retail study we've collected thousands of isolates
19 from four, five hundred samples from retail. We will be
20 testing those for the presence of TDH genes. We should be
21 able to get some estimate as to the range of densities of
22 TDH positive amongst oysters at consumption.

23 DR. MICHAEL JAHNCKE: Peggy?

24 DR. MARGUERITE NEILL: I'm wondering if you also

1 are going to use another subtyping method like PFGE. What
2 I'm thinking about is that there's data from vulnificus,
3 if I remember this correctly, that there's multiple PFGE
4 types in the oyster, but there's generally only one in the
5 patient.

6 DR. ANDY DEPAOLA: That would be correct with
7 vibrio vulnificus.

8 DR. MARGUERITE NEILL: Do we have any parallel
9 data or pending studies to answer that question for
10 parahaemolyticus? In other words, subtype the -- divide
11 them up by TDH and then PFGE them.

12 DR. NICHOLAS DANIELS: During the outbreaks in
13 New York and Texas, and at CDC, we actually did subtype by
14 PFGE, but found that serotyping correlated quite nicely
15 with PFGE. That all the 03:K6's were indistinguishable by
16 PFGE.

17 I think it is a good discriminator. I'm not
18 sure how that would add to a study. Our policy now is to
19 encourage the states to do PFGE of isolates at the states
20 and then --

21 DR. MARGUERITE NEILL: (interrupting) Human.

22 DR. NICHOLAS DANIELS: Clinical, human isolates.

23 DR. MARGUERITE NEILL: I'm talking about the
24 other way around. I'm talking about in the oysters.

1 DR. ANDY DEPAOLA: I think the PFGE may be more
2 useful as an epidemiological tool, but I don't see where
3 it's going to be that helpful in risk assessment, other
4 than if we can show that perhaps the 03:K6 is more
5 virulent and has a lower infectious dose. Then in that
6 case it may be useful.

7 DR. NICHOLAS DANIELS: Yeah. Texas did do PFGE
8 of oyster isolates and showed that those isolates were
9 different by PFGE compared to the clinical isolates.

10 DR. MARGUERITE NEILL: Not to belabor the point,
11 but the oyster isolates are TDH negative.

12 DR. NICHOLAS DANIELS: Yes.

13 DR. ANDY DEPAOLA: What little data is available
14 among the TDH positive isolates there's a lot of
15 variability in molecular fingerprinting techniques. This
16 is from some work that we've done with Steve Gentle
17 (phonetic). A lot of that is from Asia. But none that
18 says that there's one particular PFGE that has a lower
19 infectious dose.

20 DR. MICHAEL JAHNCKE: Other questions. Please
21 remember to identify yourself when the questions are
22 asked, and even responded to. Bob?

23 DR. ROBERT BUCHANAN: Bob Buchanan, FDA. First,
24 I'd like to commend the group for an obviously tremendous

1 amount of work that's been done since we last met at the
2 last Advisory Committee.

3 Second, I'd like to explore a little bit more in
4 the -- Marianne, in your final statement you indicated
5 that you are going to be done simulation modeling. Have
6 you thought of on what types of distributions you're going
7 to be assuming at the different phases of your evaluation,
8 and in a lack of having what appears to be a tremendous
9 amount of data, you have a very few limited studies, if
10 you must default to a specific distribution to assume how
11 you're going to handle that choice?

12 DR. MARIANNE MILIOTIS: As I said, this is a
13 very -- this is a preliminary model and we haven't quite
14 got to that stage yet. We're just trying to see how we
15 can integrate all the data that we are picking up. The
16 data we do have is the temperature of the salinity in some
17 of the data from Nick Daniels.

18 DR. MICHAEL JAHNCKE: I have a question. What
19 is your -- for the data that you do have what are your
20 assumptions or your criteria that you're using to either
21 accept and use that data, or reject that data? Does it
22 vary depending on which part of the assessment you're
23 doing? I'm just asking what your base assumptions and
24 criteria are for accepting published data, or not.

1 DR. CHARLES KAYSNER: Chuck Kaysner, FDA. There
2 is very limited published. There is quite a bit of
3 unpublished data in my laboratory and Andy DePaola has
4 quite a bit that hasn't been published too. But,
5 basically for pre-harvest module there was a relatively
6 low amount of published data. Most of it -- I think in
7 the late seventies is the last time we saw any.

8 DR. MICHAEL JAHNCKE: I guess my question is
9 even on the published data what criteria are you using --
10 I mean, not all published data may be useable. My
11 question is what is your basic criteria for accepting
12 published data and using it in your risk assessment or
13 saying that it is not appropriate, or incorrectly done, or
14 however you want to put it, but rejecting it?

15 DR. MARIANNE MILIOTIS: I think as Chuck said,
16 since this is limited data, we have limited published
17 data, we will take everything we can get, as long as it's
18 pertinent to the risk assessment and it will help in our
19 risk assessment.

20 DR. ANDY DEPAOLA: In the case of the post-
21 harvest there is no published data, so we don't have to
22 worry about throwing out too much.

23 DR. MICHAEL DINOVI: Yeah, and from my point of
24 view, as I mentioned briefly, things have to validate out.

1 I can't produce a model that suggests we're eating a
2 million pounds of oysters when there's a hundred million
3 pounds sold.

4 So at the end I feel my section will need to
5 have definite corroborative data probably from the
6 industry. But that's probably the best source.

7 DR. MICHAEL JAHNCKE: Bob?

8 DR. ROBERT BUCHANAN: Bob Buchanan, FDA. To
9 follow that up a little bit further, one of the nice
10 things that was available, for example, with the risk
11 assessment that was done on salmonella in eggs was that
12 the end prediction they could then compare against CDC's
13 data on the yearly outbreak rate.

14 Do you have anything that you see on the horizon
15 that you can use to validate your estimates, or at least
16 determine whether or not you're going to be in ballpark
17 when you come up with an answer?

18 DR. MICHAEL DINOVI: Yes. We actually saw one
19 small bit, the data from Florida, suggesting on their
20 rates, and Marianne's data and other epidemiological data
21 you already have -- and CDC data, you have numbers of
22 illnesses. We have -- I mean, you can do arithmetic on
23 approximate total intakes, population-wise. You can
24 certainly gin up a crude number without really using any

1 of this, but that doesn't really get you anywhere.

2 The reason to break all this out and build up
3 all the variability and uncertainty is so that you can
4 then go take it apart and see what's important.

5 One of the things that John Bowers will
6 certainly have to do is sensitivity testing on all this.
7 It may turn out that sections we have very little data on
8 don't effect the outcome anyway. So you don't want to
9 spend time and money on that. So yeah, we will have to do
10 all these things. Certainly at the next meeting John
11 Bowers will be out in front showing you the actual
12 numerical models and how they effect the total.

13 Again, you go back to the first thing, yes, we
14 will have -- you can't say you're going to make ten
15 million people sick when you only have five thousand
16 cases.

17 DR. ROBERT BUCHANAN: A couple other points on
18 that. I guess this would be directed towards some of the
19 researchers that were telling us about their future plans.
20 While there's a cutoff date of July 6 that Marianne talked
21 about, if the final risk assessment is going to be
22 completed around November, are any of your future studies
23 going to be completed about that time, that you could
24 actually go back and validate your predictions against the

1 data that you're achieving? I guess I'm directing that to
2 Andy.

3 DR. ANDY DEPAOLA: This is Andy. The problem
4 with the cooperation with the states is that some are
5 going to be ready to begin earlier and some are going to
6 begin later. So there will be some data, but it won't be
7 complete. There will be data from Alabama since March and
8 I don't know that any of the other states have started
9 their sampling. We have the data Jan Guch has collected
10 from Alabama over the last year. Some Galveston Bay data
11 that may be a little bias because it was after an outbreak
12 last year. I think the State of Washington has quite a
13 bit of data from the last two years.

14 So, there should be more available date in
15 November, but I think there will still be a fair amount of
16 uncertainty.

17 DR. MICHAEL JAHNCKE: Bob?

18 DR. ROBERT BUCHANAN: Again, Bob Buchanan, FDA.
19 I'll ask the same question of CDC. Are there any plans
20 under food net to be examining vibrio data and if so, will
21 any of it be available about the time that this risk
22 assessment is completed?

23 DR. NICHOLAS DANIELS: Currently through food
24 net we're trying to come up with estimates of disease

1 burden for a variety of pathogens, and vibrio is one of
2 them. So we may have some estimates. Currently Paul Mead
3 is working on that, so we may have that by November.

4 DR. DONALD BURR: This is Don Burr from the FDA.
5 Certainly it's not going to be finished in time, but the
6 FDA is supporting some work again by Glen Morris, where
7 he's actually taking the surrogate organism, the non-01
8 vibrio cholera and trying to spike oysters with that, and
9 actually feeding those to humans. Then trying to couple
10 that with animal models, the RITARD model, whatever they
11 come up with, to see if they can do some extrapolations
12 with dose-response. It's not going to be there on time,
13 but there is some of that data that's going to hopefully
14 be generated in the future.

15 DR. MICHAEL JAHNCKE: Other questions? Bob?

16 DR. ROBERT BUCHANAN: I was just going to say as
17 a general comment, certainly if you can get that kind of
18 data not long after the risk assessment is finished, and
19 it matches up, it certainly is a very strong statement
20 about the effectiveness of this tool.

21 DR. MICHAEL JAHNCKE: Other questions, comments?
22 Morrie?

23 DR. MORRIS POTTER: Again we have an opportunity
24 for comment from the gallery. Any non-committee members

1 who would like to present information or opinion are
2 welcome to at this time, or to submit information to the
3 written record until that closes.

4 Dane, did you have something?

5 MR. DANE BERNARD: Yes, thank you. Dane
6 Bernard. There was one other question that bothers me a
7 little bit, because the disease data that's been collected
8 shows both wound source and food ingestion source. As you
9 go through and validate the model I'm assuming we're
10 focusing solely on ingestion as the vehicle. We're going
11 to tease out those sources, I would trust.

12 DR. MARIANNE MILIOTIS: This is Marianne
13 Miliotis. Yes, we are. We are just focusing on
14 consumption. We're not looking at wound infections or any
15 other kind of infection.

16 MR. DANE BERNARD: So as we go through here and
17 we look at the potential impact of interventions and the
18 benefits and the costs of doing those, we'll be focusing
19 solely on that portion exclusively.

20 DR. MARIANNE MILIOTIS: On consumption of raw
21 molluscan shellfish, yes.

22 DR. MORRIS POTTER: Morris Potter here. Dane,
23 the data collected by CDC in collaboration with its
24 partners and other federal agencies and state agencies

1 through the Gulf Coast Surveillance and Food Net, we will
2 be looking at the distinction of food versus non-food
3 sources.

4 So, the epidemiologic data that will be
5 collected will serve as a barometer for how well we're --
6 how well the risk assessment matches observed disease.

7 Again, FDA is taking this risk assessment very
8 seriously. We would very much appreciate data coming from
9 all sources, and people taking advantage of data that
10 exists in various forms to answer the five questions that
11 were written in the Federal Register announcement.

12 I see Spencer's hand, Spencer Garrett.

13 MR. SPENCER GARRETT: I'm Spencer Garrett from
14 the National Marine Fishery Service, one of the sponsoring
15 agencies of NACMIF, and let me say I want to echo Bob
16 Buchanan's job well done to you folks for giving us a
17 day-long presentation on vibrio parahaemolyticus certainly
18 in depth and scope.

19 In my agency risk assessment, just the phrase
20 risk assessment, is one of the most misunderstood things
21 that we deal with. Remember, we're primarily a fishery
22 management agency. We deal with food safety issues pre-
23 harvest and we do risk assessments to try to mitigate, if
24 you would, or prevent over fishing. It's kind of like a

1 little bit different twist. Actually, it's kind of like
2 the opposite twist, if you think about that for a moment
3 or two.

4 But, risk assessment itself from a public health
5 perspective has a very finite meaning. At least I've been
6 told it has a finite meaning.

7 Internationally at least, and nationally this
8 committee has put out a publication on risk assessment.
9 International the CODE X Elementarious Commission is going
10 to be adopting just this year at the commission meeting a
11 document entitled, "The General Principles and Guidelines
12 for the Application of Microbiological Risk Assessment."

13 For all of you who have perhaps not read that
14 document, and I realize you have a very large risk
15 assessment team here, so some probably have and some
16 perhaps have not, I would urge you to read that, because
17 it does give a general road map that one can follow and
18 then be on very good defensible grounds in terms of the
19 transparency of the process, but also recording all of the
20 assumptions that you make.

21 Just some general comments I'd like to make
22 myself. One, I also agree with Bob that I think that you
23 have to engage in multi endpoints, not just one endpoint.
24 I think you do have to talk about the sub-populations at

1 risk, and discriminate that statistical probability
2 against the population perhaps that are not at risk.

3 As a matter of fact, I think the Federal
4 Register Document indicates that you're going to do that.
5 We would hope that you would do that.

6 Secondly, I'm not sure personally myself about
7 the necessary time. I see there are some data gaps, and
8 there are always data gaps when you do things. I
9 certainly understand that. I'm not certain what's drawing
10 the time line.

11 Finally, this goes to the consumption model.
12 There was a paper published, I think we may have helped
13 finance it, by TECHWE (phonetic) in 1985, called the
14 "Social Economical Profiles of Oyster Consumption in the
15 United States."

16 In that particular paper they stratified the
17 oyster consumption by pounds eaten at home, away from
18 home, seasons, you know, summer, fall, winter spring, race
19 being White, Black, and other. Urbanization being whether
20 the oyster eaters were from an urban setting, a suburban
21 setting, or a non-metro setting. Education levels, just
22 like some of the studies that you quoted, but also per
23 capita income. It might be interesting to get a copy of
24 that study and then bounce that against some of the later

1 studies that have gone on.

2 From our agency's perspective obviously we'll be
3 following this very closely. If you would like this study
4 we can formally submit this study that's been published to
5 FDA to have it in your quiver so to speak.

6 We wish you well. It's going to be a very
7 challenging task, I can assure you. Thank you.

8 MS. ROBIN DOWNEY: My name is Robin Downey, I'm
9 with the Pacific Coast Shellfish Growers Association. I'm
10 going to make a few very brief comments. I'm actually
11 making them on behalf of the entire shellfish community,
12 with the Molluscan Shellfish Institute, and the Louisiana
13 Oyster Task Force, and the Gulf Oyster Industry Council.
14 We have all been meeting together and talking about this
15 issue for some time.

16 I'd like to state a few obvious points.
17 Obviously, we all have been talking about this. There are
18 several studies right now that are being conducted on this
19 issue. It does seem a bit premature to go ahead and try
20 to ramrod this whole process in this risk assessment
21 before all that information is in. I know that there are
22 some political things that are kind of driving this, but I
23 would urge this committee, if at all possible, to hold
24 this off for another year until that information is in.

1 I'm curious as to why there's been so much
2 information about vulnificus included in the information
3 today. I do urge you to keep vulnificus and
4 parahaemolyticus very much apart from each other.

5 I'm also concerned that the differences between
6 the different coasts have not really come forward today in
7 the information that's been presented. I would be happy,
8 as a representative of the West Coast to provide
9 information to this committee where you are lacking
10 information.

11 For example, when the information was provided
12 on harvesting and processes for harvesting, what was
13 presented is not indicative of what we do on the West
14 Coast at all.

15 Some of the statistics that were provided in
16 this information forum today were somewhat inaccurate.
17 I've talked to a couple of you about that. I'll be happy
18 to provide that information.

19 I think the whole shellfish community can say
20 that we'd be happy to provide that information.

21 Also, there was no differences really noted in
22 commercial versus recreational illnesses. Washington
23 State does have that information. I urge you to please
24 separate those two issues. They are different.

1 With all that said, I would just like this
2 committee to keep in perspective food-borne illnesses and
3 that shellfish illnesses really constitute a very small
4 percentage of what food-borne illnesses happen in this
5 country.

6 However, we're happy for the work that's being
7 done here. I think it's very necessary. We certainly
8 want to keep our consumers healthy. I'd like to thank all
9 of you for the work that you will be doing.

10 DR. MORRIS POTTER: Thank you very much for
11 those comments, and for the offer for information. We
12 would be very anxious to have as much information on
13 industry practices and consumption data as we can get to
14 make the risk assessment that we do as correct and robust
15 as possible.

16 As far as why now and why not a year from now.
17 A year from now there will also be unanswered questions
18 and more data in the offing. I think once we have a risk
19 assessment framework developed, inserting or replacing
20 data points or distributions in the model becomes a bit
21 easier. I'm not sure that we lose a lot by moving ahead.
22 But, thank you very much for the offer. We will be
23 anxious to see what information you can share.

24 Dane?

1 MR. DANE BERNARD: Thank you. Dane Bernard.
2 The commenter brings up a couple of good points. I agree
3 with Dr. Potter's comment. To move ahead puts together a
4 framework that allows us to plug in data as it comes along
5 and refine our output.

6 I think the place to use caution is once we get
7 to a point of having some output of some kind from the
8 risk assessment. Do we have enough confidence in that
9 output to make decisions? I think at that point one
10 should look at what data is in the pipeline, what studies
11 are close enough to then say can we go ahead and make a
12 decision or should we wait on making policy decisions.

13 I think the point that was made is that there
14 may be some additional data which will allow us to make
15 better policy decisions, and it's at that point that we
16 need to think about the risk management decisions that we
17 make as a result of the output in light of the closeness
18 of additional information.

19 Also, I think we need to be very clear once we
20 get to the point of having some output as to the degree of
21 confidence we have in the output, the level of
22 uncertainty. One of the problems that I see with certain
23 risk assessments is that while the uncertainties involved
24 in the estimates are in the reports, they're not usually

1 up-front. You have to dig through the reports. I
2 personally think that we need to be more clear about how
3 accurate our predictions are and what the range of
4 uncertainty is so that once we put out a report it's clear
5 as to what its limitations are.

6 DR. MORRIS POTTER: Thank you for that counsel,
7 Dane. Cathy?

8 MS. CATHY DONNELLY: Cathy Donnelly. I'm
9 wondering if the CDC has any plans to do a population-
10 based active surveillance for this pathogen. It seems
11 with the increase in cases over recent years, coupled with
12 the fact that about 88 percent of the cases involve
13 hospitalization, it seems it would be well set up for that
14 type of study.

15 DR. NICHOLAS DANIELS: We're doing active
16 surveillance in our food net sites, and vibrio is
17 infection that we do have surveillance for. We'll have
18 data from that.

19 MS. CATHY DONNELLY: Will that also include
20 going back to the patient's home and pulling food samples?

21 DR. NICHOLAS DANIELS: Usually if there's a
22 culture-positive case in a food net site, that patient is
23 interviewed and it includes sort of a trace-back of where
24 those oysters were harvested, how many that person ate,

1 how they consumed the oysters. So we do get a lot of
2 information from that. We are asking food net sites to
3 forward isolates to us for subtyping.

4 DR. MORRIS POTTER: Peggy?

5 DR. MARGUERITE NEILL: Peggy Neill. I was just
6 going to raise one issue about the food net surveillance
7 particularly for this organism, because as bad as it was
8 for the lack of culturing by so many laboratories for e-
9 coli 0157, I mean, it's in orders of magnitude greater for
10 vibrio. I'm in Rhode Island, which is a coastal state.
11 Massachusetts, Rhode Island, and Connecticut have probably
12 less than ten percent of the labs that are routinely with
13 culture media on hand for the detection of vibrio.

14 So therein, I think, lies the real difficulty to
15 look toward food nets for that answer, even though it's in
16 a position to give it, if the supporting labs were doing
17 routinely.

18 DR. NICHOLAS DANIELS: We did do a laboratory
19 survey in the food net sites and found that around 20
20 percent of labs routinely use TCBS, but again, that's a
21 small fraction of the labs in food net.

22 DR. MORRIS POTTER: Other comments? Around the
23 table? Committee away from the table? Others? Guests?
24 No parting shots?

1 Thank you all very much. I'd like to thank the
2 Risk Assessment Task Force for doing a very good risk
3 assessment start here, and for making a very nice
4 presentation.

5 I'd like to thank the committee for its
6 forbearance and all of our guests for joining us today.

7
8 (Whereupon, the proceedings in the above-
9 entitled matter were concluded at 4:10 p.m.)
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STATE OF ILLINOIS)
)
COUNTY OF C O O K) SS.

I, ANNE I. MAZIORKA, CERT, a Notary Public
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That the foregoing transcript was reported to me
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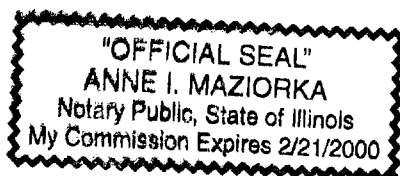
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IN WITNESS WHEREOF, I do hereunto set my hand
and affix my seal of office at Chicago, Illinois, this

11th day of June, 1999.



Anne I. Maziorka
ANNE I. MAZIORKA, CERT
Notary Public, Cook County, IL

My Commission expires 2/21/2000.

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